To analyse the impact of Zn and Cu nanoparticles through green synthesis on okra .

Research protocol

For Registration in Ph.D. Program

Submitted by

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Dated : ……

CERTIFICATE

This is to certify that this research protocol entitled **"To analyse the impact of Zn and Cu nanoparticles through green synthesis on okra."** by **DEEPA PANDEY**, submitted in fulfilment of the requirement for registration in Ph.D. Program in Department of Botany under School of Science, JECRC University, Jaipur, during the academic year 2023-2024, is a bonafide record work carried out under my guidance and supervision.

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1. INTRODUCTION

Meeting the dietary requirements of the rapidly growing global population is imperative in the present circumstances. Approximately 33% of conventionally produced crops experience harm, due to the presence of pests, microbiological attacks, adverse weather events, suboptimal soil conditions, and limited access to essential nutrients. In order to address these issues, there is a pressing need for the development and implementation of innovative technologies. The agrotechnological revolution, which is poised to transform the existing agricultural system and provide food security, has been facilitated, to some extent, by the integration of nanotechnology.

Consequently, nanoparticles are undergoing advancements as a state-of-the-art substance that has the potential to revolutionize contemporary agricultural practices.

A comprehensive understanding of the interaction between plants and nanomaterials presents novel opportunities for improving agricultural practices through the augmentation of features like as disease resistance, crop yield, and nutrient absorption (Mittal *et. al*., 2022).

Ensuring food security in emerging nations poses considerable challenges due to various factors, including “low agricultural sector productivity, depletion of natural resources, substantial post-farming losses, little value addition, and rapid population expansion”. Scholars are currently engaged in efforts to integrate contemporary technologies in order to enhance the supply chain and mitigate the disparity between food supply and demand. Nanotechnology is regarded as a very promising field that holds the potential to enhance agricultural productivity by means of efficient pesticides and herbicides, soil characteristics modulation, wastewater management, and disease detection. The utilization of nanoparticles for the purpose of prolonging the duration of shelf life also holds potential for mitigating losses incurred during the agricultural process. Further investigation is required in order to effectively mitigate the safety and health concerns related with the aforementioned technology (Neme *et.al*., 2021).

The root of the radish plant, Raphanus sativus, an annual or biennial member of the Brassicaceae family, is grown for human consumption. (Britannica, 2023).

Researchers focused primarily on the nutrients and phytochemicals in roots and leaves. Flavonoids accounted for 38.8% of the data, making them the most prevalent group. “Non-flavonoid polyphenols came in second with 8.4%, followed by terpenes and their derivatives with 8.2%, fat and compounds related to fat with 6.4%, and glucosinolates and their breakdown products with 5.6%. High levels of macronutrients, calcium, potassium, sodium, fibre, fatty acids, and non-flavonoid polyphenols were found in leaves. (Gamba *et.al., 2021)*. In a study, The leaf extract was subjected to phytochemical analysis, which identified alkaloids, flavonoids, glycosides, tannin, phenolic compounds, triterpenoids, and steroids in addition to detecting the presence of photosynthetic pigments (3.40 mg/g fresh weight), ascorbic acid (0.000429 mg/g fresh weight), and foliar phenol content (0.0270 mg/g fresh weight)”. (Agarwal, K. and Varma, R., 2014)

Metal ions can be converted to nanoparticles using biomolecules found in plant extracts in a single step of green synthesis. This naturally occurring process of converting a metal ion to a base metal can be easily scaled up, conducted at room temperature, and occurs quite quickly. Plant extracts are used in environmentally friendly synthetic processes. The various water-soluble plant metabolites (such as alkaloids, phenolic compounds, and terpenoids) and co-enzymes are the involved reducing agents. Plant-based syntheses have been especially focused on producing silver and gold nanoparticles. It has been successful to create nanoparticles using extracts from a wide variety of plant species.

Chemical and engineered nanoparticles (ENPs) are now being used more and more by researchers in fertiliser and pest control applications. However, researchers are using a more eco-friendly and biological method of synthesis of nanoparticles to address the ecotoxicological effects of chemical fertilisers and ENPs on the soil system. The use of biological extracts, which come with natural capping and reducing agents, is thought to be safe, economical, and comes from a variety of plants and microorganisms. These extracts include phytochemicals like ascorbic acid, proteins, and polyphenols. In order to create nanoparticles in a sustainable manner, scientists are actively investigating “various plant extracts, flowering and fruiting parts, microbial components, cellular components, and more”. (Senthamarai and Malaikozhundan, 2022).

In both plant and human metabolic processes, zinc ions (Zn^{2+}) play a significant role as an essential trace element. Zinc oxide nanoparticles (ZnO NPs) have recently attracted a lot of attention due to the Food and Drug Administration (FDA) confirmation that they are safe.

The reason for all this interest is that plants need more zinc to grow properly, and many soils don't have enough of it (Itroutwar *et.al.,* 2020). According to Laware and Raskar (2014), all living things require the micronutrient zinc (Zn). Plants need to synthesise proteins, carbohydrates, phytohormones (such as auxin), membrane function, seedling vigour, photosynthesis, and defence against biotic and abiotic stress (Laware and Raskar, 2014). Thus, a deficiency in zinc causes a plant to have smaller leaves, narcotic leaves, stunted growth, and low-yielding, low-vigor seeds (Elhajand Unrine 2018).

Scientists have started using zinc oxide nanoparticles as foliar fertilizers, which means they apply these tiny particles directly to the leaves of plants. This practice helps improve various aspects of plant growth, like their appearance, how they use sunlight to make food (photosynthesis), how much plant material they produce (biomass), and in the end, how much crop they yield (Munir, 2018).

Copper (Cu), a microelement found in block D and period 4 of the periodic table, is essential for the growth of plants. Under physiological circumstances, it exists as Cu^{2+} and Cu^{+}. Curled leaves, bent petioles, light chlorosis, and a permanent loss of turgor in young leaves are all signs of a copper deficiency in plants. Chronic copper deficiency causes rosette-shaped growth. [Harold *et.al*. (2007), Inmaculada yruela, (2005), A. Manceau *et.al.* (2008)]. Plants require the metal copper to survive. It is essential for defence against oxidative stress, ethylene sensing, cell wall metabolism, photosynthetic and respiratory electron transport chains, and molybdenum cofactor biogenesis. Therefore, critical plant metabolic processes may be impacted by a copper deficit. Yruela, I. (2009). In the near future, copper deficiency might be more common. To resolve these problems in crop production green synthesized Cu nanoparticles, play an important role.

“Vegetables are plant parts that are eaten as food by people and other animals. The original definition is still frequently used to refer to all edible plant material, including the flowers, fruits, stems, leaves, roots, and seeds, when applied to plants as a whole. A different definition of the term is frequently used arbitrarily, according to cultural and culinary traditions. Vegetables, which are typically low in fat and carbohydrates but high in vitamins, minerals, and dietary fibre, can be consumed either raw or cooked and are crucial to human nutrition. Numerous nutritionists advise eating five or more portions of fruit and vegetables each day, as recommended by many of them”.

Okra, or *Abelmoschus esculentus* (L.) Moench, is cultivated all over the world in tropical and subtropical climates. It belongs to the Malvaceae family. It is commonly known as 'Bhendi' or 'Ladies finger' and is one of the most widespread and commercially grown vegetables in tropical countries (Singh et al., 2014; Sandhi *et.al*., 2017). Okra cultivation covers 5.13 lakh hectares (ha) in India, with a production of 61.70 lakh metric tonnes (MT) and a productivity of 12.03 MT/ha. Okra is grown on 0.64 lakh ha in Odisha, with a yield of 5.66 lakh MT and a productivity of 8.85 MT/ ha (Horticultural Statistics at a Glance, 2018).

2.Review of Literature

Some previous studies have been reported that metal oxide nanoparticles are effective for crop improvement in different modes. Some of these are being described below-

The successful synthesis of ZnO:CuO and ZnO nanoparticles using plant extracts as capping agents is demonstrated by Vibitha *et.al.*(2020). Pansambal *et.al.* (2017) also demonstrates the environmentally friendly synthesis of CuO nanoparticles using different plant extracts. Umavathi *et.al.* (2021) made ZnO nanoparticles using *Parthenium hysterophorus* leaf extract, further highlighting their antimicrobial and growth-promoting qualities.

As an alternative to antibiotics and chemical fungicides for crop disease management, Rathore *et.al.* (2018) highlights the antimicrobial properties of CuNPs synthesised from microbes and plant sources. According to Nguyen *et.al. (*2022), CuNPs created under microwave irradiation using green agents have antifungal activity against pathogenic fungi on plants. In Imtiaz *et.al. (*2022), it is discussed how plant extracts can be used in green synthesis techniques to create nanoparticles with special physiochemical properties that can improve plant yield and growth. Ortega-Ortez *et.al. (*2022) focuses specifically on copper oxide nanoparticles (CuONPs) biosynthesised from orange peel, demonstrating that low doses of CuONPs enhance germination, seedling development, and phytochemical compound production in wheat sprouts.

Awadh *et.al.* (2022) conducted experiment and synthesized green NPs of zinc oxide (ZnO) by using *Raphanus sativus* leaf extract. The size of ZnO NPs was determined by XRD.

Rastogi *et.al.* (2017), conduct experiments to analyse the impact of different metal and metal oxide nanoparticles on plant.

Nagar, Niharika and Devra, Vijay. (2018)., synthesize green nanoparticles of Cu using *Azadirachta indica* leaves. Several instrumental techniques were used to characterise the biosynthesized CuNPs. Plant biomolecules serve as stabilising and capping agents in addition to inducing the reduction of Cu^{2+} ions to CuNPs.

Alabdallah *et.al.* (2020), conducted research to analyse the effect of green synthesized ZnO NPs or ZnO on the progress of cowpea and okra under six different salt stress conditions. They used to foliar spray after (20,40,60 days) from sowing. They observed that application of green synthesized ZnO NPs increase the growth parameters as compared to ZnO and control.

In okra plants grown in saline conditions, Zafar *et.al. (*2021) discovered that foliar application of Zn-GNPs increased shoot and root weight, chlorophyll content, and antioxidant activity. Furthermore, TaheriMelika *et.al.* (2015) showed that maize plants' shoot dry matter and leaf area index improved when ZnO nanoparticles were added to irrigation water. These results suggest that Zn nanoparticles can effectively reduce the detrimental effects of salt stress on plant growth, including in okra plants.

Gui et.al., (2023) discovered that the nutritional value of maize and soybean plants was impacted by species-dependent interactions between CeO2 nanoparticles and Ce3+ ions. Khattab et.al., (2023) showed that spraying turmeric plants with ZnO nanoparticles increased growth, yield, and curcuminoid content. According to Dola et.al., (2023), soybean plants' growth, yield, and ability to withstand drought were all improved by foliar spraying with nano zinc (ZnO). Patil et.al., (2023) did not specifically address the impact on crops and instead concentrated on the physicochemical characteristics of ZnO nanoparticles.

Overall, the above review of literature suggests that the use of Zn and Cu nanoparticles may improve crop growth, yield, and nutritional quality, though the precise effects may vary depending on the type of crop and the nanoparticles employed.

3.Research gap

Zinc (Zn) and copper (Cu) nanoparticles, synthesized through green methods, have demonstrated positive effects on various crops. Effect of Zn NPs on okra crops has been observed under salt stress conditions. However, the impact of radish leaves on okra has not been previously observed. In some research, zinc oxide nanoparticles (ZnONPs) were synthesized using radish leaf extract, but they were not tested or applied to any crops.

Surprisingly, there have been no reported studies on the synthesis of Zn and Cu nanoparticles using radish leaves (specifically, Akra Nishant) and their application in the agriculture sector. Also, there is no studies related to the application of ZnONPs, synthesized using radish leaves extract, on the selected vegetable crop (okra) have been found. There has been a lack of research evaluating the effects of these nanoparticles on the quality of vegetables (minerals and other nutritional parameters).

The proposed study **"**To analyse the impact of Zn and Cu nanoparticles through green synthesis on okra**"** will be done first time ever.

4.Objectives

The proposed research will be carried out to fulfil following objectives-

1. To synthesize and characterize Zn & Cu nanoparticles (NPs) by using extract of radish leaves.
2. To evaluate the effect of the synthesized green NPs on seed germination parameters of Okra with comparison to commercialized (already available in market) NPs.
3. To analyse the effect of the synthesized green NPs on morphological & yield parameter of Okra.
4. To analyse the impact of the synthesized green NPs on biochemical parameters of Okra crop.

5. Methodology

Synthesis of ZnO nanoparticles

In order to produce ZnO Nanoparticles, a solution of 0.02 M zinc nitrate hexahydrate will be created, consisting of 90 ml. This solution will then be subjected to heating at a controlled temperature of 30 °C ± 2 °C for a duration of around 10 minutes. The addition of radish leaves extract should be carried out gradually and with constant stirring. The solution will then be allowed to settle for a duration of 2 hours, during which the precipitate will accumulate at the bottom. The solution will subsequently undergo centrifugation for a duration of 10 minutes. The pellets will be gathered and subjected to a washing process using deionized water. The detection of zinc oxide nanoparticles can be inferred from the

production of a precipitate with a creamy appearance. Subsequently, the final product was subjected to overnight drying in a hot air oven (Javed *et al*., 2022).

Synthesis of Copper nanoparticles

The procedure will be similar to ZnO. Instead of zinc nitrate Copper (II) Sulphate pentahydrate ($CuSO_4.5H_2O$) will be used as Cu source.

Characterization of the synthesized green nanoparticles

The synthesized nanoparticles will be characterized for their size, shape, purity by –

- UV (Ultraviolet)
- FTIR (Fourier transmission infrared spectroscopy)
- FESEM (Field emission scanning electron microscopy)
- EDX (Energy dispersive X-ray spectrometer)
- XRD (X-ray diffraction)

Evaluation of effects of nanoparticles on seed germination parameters

Seeds of okra will be surface sterilized and soaked in different concentrations of the prepared nanoparticles (concentration will be decided later) and those were kept on filtered papers in petri dishes separately for germination. Besides these green synthesized nanoparticles, seeds treated with commercialized NPs and also be kept for germination along with control (water only).

The seed germination percentage, number of days to seed germination, total seedling length, shoot length, root length, fresh weight, dry weight will be measured.

After seed germination test, the best concentration range for germination will be decided for pot experiment.

Pot experiment

Characterized soil will be used for the experiment. Seeds (okra) will be soaked in soil and treatment will be given for different concentrations (the concentration will be decided on the basis of results of Petri plate experiment). Concentration of the synthesized NPs as well as reference nonfertilizer.

Determination of Morphological parameters

Plant height, total number of branches/ plants, total number of leaves/branches after 30,60 and 90 days.

Day to flowering, number of flowers at different days etc.

Yield parameters

Number of fruits per plant at different days, fruit /plant and fruit weight at 30,60 and 90 days.

Biochemical parameters

Determination of total flavonoid content (Kaviarasan *et al*., 2007), total phenolic content (Ghasemzadeh *et al*., 2010), total carbohydrates (Hedge and Hofreiter, 1962), total proteins (Lowry *et al*. 1957), total lipids (Folch *et al*. (1957).), fibres ((Raghuramulu *et al*., 2003), minerals (Raghuramulu *et al*., 2003) etc. will be determined in fruits (as these crops are grown for their fruits mainly) using their standard methods.

Note*: all the experiments will be conducted in triplicates and statistical analysis will be done to know the presence/absence of significance variations among various treatments.

Instrumentation used

For the whole study, following instruments will be utilized-

- Weighing balance
- Magnetic stirrer
- Sonicator
- Hot air oven
- Hot plate
- Incubating shaker
- UV spectrophotometer
- FTIR (Fourier transmission infrared spectroscopy)
- FESEM (Field emission scanning electron microscopy)
- Centrifuge
- Laminar air flow

- Autoclave etc.

Details of External Resources

For characterization of the synthesized nanoparticles, facilities of MRC, MNIT, Jaipur, and Biomitra Life Sciences Pvt. Ltd., Jaipur will be utilized.

6. Expected outcome

The salts for nanoparticles will be purchased from standard company. Radish leaves will also be collected from the local vegetable market. Nanoparticles will be synthesized using quality standards. Characterization will be done at laboratory recognized by Indian Standards. During all experiments either petri plates/ pot culture/ biochemical evaluation, all standards will be followed.

7. Year-wise work plan

First year:

- Synthesis and characterization of both kinds of nanoparticles.
- Petri plate experiment and to evaluate the effects of nanoparticles on seed germination parameters.

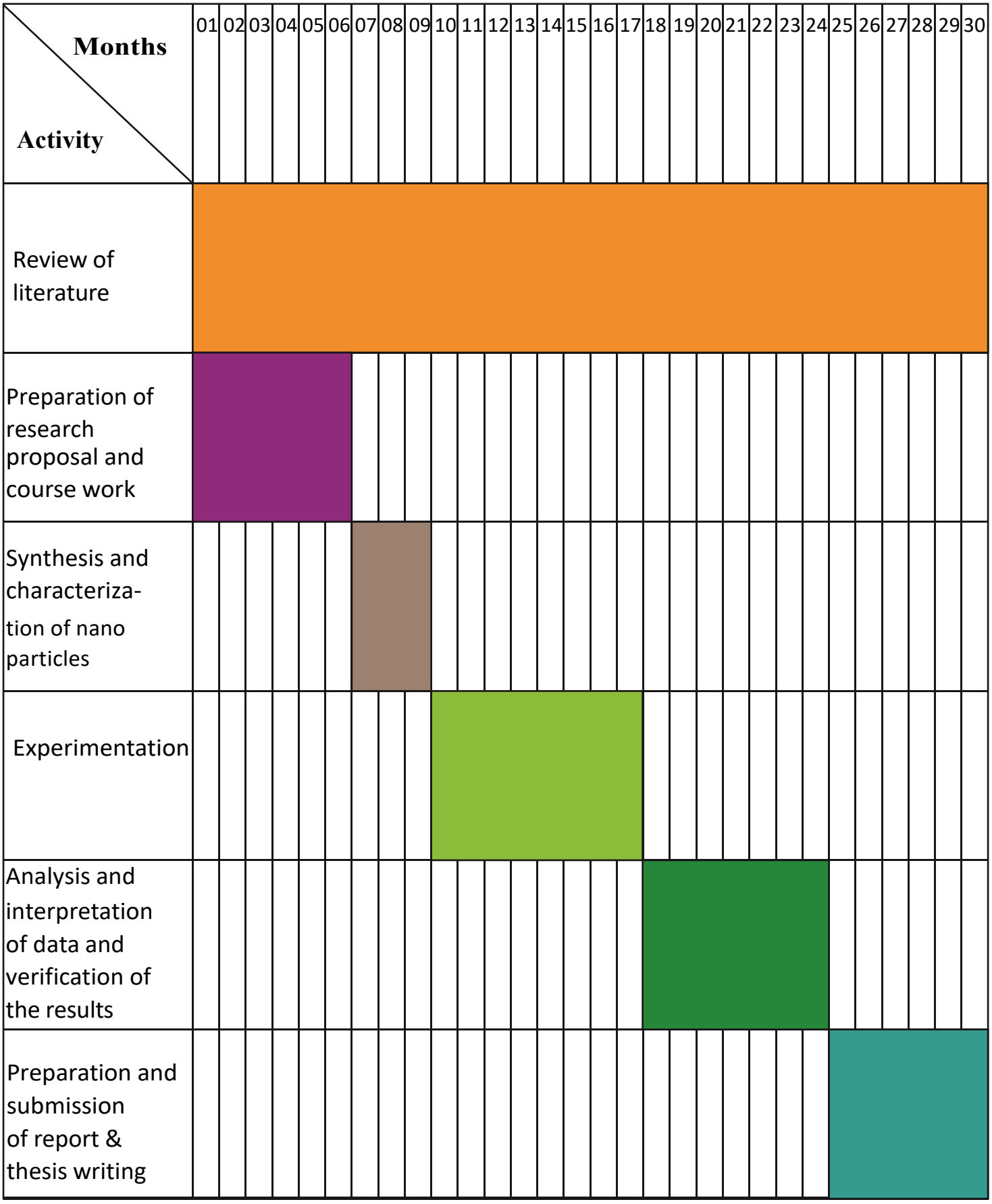
Second year:

- Pot experiment for okra crop
- Morphological parameters observation
- Yield parameters observation
- Collection and processing of fruits for biochemical assay.

Third year work plan:

- Calculations and statistical analysis of the observed data
- Remaining tenure will be used for writing research papers and thesis.

WORK PLAN (Months Wise)


Months
Activity
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Review of literature
Preparation of research proposal and course work
Synthesis and characteriza-tion of nano particles
Experimentation
Analysis and interpretation of data and verification of the results
Preparation and submission of report & thesis writing

8. Significance of the proposed study

Radish leaves come in bulk during winter time. A good amount of radish leaves is destroyed or rot due to an excess number of leaves. So, we are able to use the leaves so efficiently. A lot of beneficial elements and other organic compounds are found in radish leaves which are beneficial for the okra crop. NPS of Radish leaves has slow nitrogen release due to which okra crop gets same amount of nitrogen and other components. If the study goes positive, farmers and vegetable sellers would be able to manage excess amount of radish leaves, more effectively and also improving the quality of okra crops. This dual benefit can lead to cleaner and more productive farming practices.

9.Limitations

I will be working exclusively with the Jaipur variety of okra and radish leaves.

10. References:

A. Manceau, K.L. Nagy, M.A. Marcus, M. Lanson, N. Geoffroy, T. Jacquet and T. Kirpichtchikova. (2008). Formation of metallic copper nanoparticles at the soil-root interface, Environ Sci Technol., 1(5), 1766-72.

Agarwal, K., Varma, R. (2014) Radical scavenging ability and biochemical screening of a common Asian vegetable - Raphanus sativus L. *International Journal of Pharmaceutical Sciences Review and Research Volume 27, (Issue 1) Pages 127-134*

Al Awadh, A. A., Shet, A. R., Patil, L. R., Shaikh, I. A., Alshahrani, M. M., Nadaf, R., Mahnashi, M. H., et al. (2022). Sustainable Synthesis and Characterization of Zinc Oxide Nanoparticles Using Raphanus sativus Extract and Its Biomedical Applications. *Crystals, 12*(8), 1142. MDPI AG. Retrieved from http://dx.doi.org/10.3390/cryst12081142

Alabdallah N. M, Alzahrani H. S. (2020). Impact of ZnO Nanoparticles on Growth of Cowpea and Okra Plants under Salt Stress Conditions. *Biosci Biotech Res Asia*;17(2)

Britannica, T. Editors of Encyclopaedia (2023, August 24). *radish*. *Encyclopedia Britannica*. https://www.britannica.com/plant/radish

Brown RC (ed) (2003) Biorenewable resources—engineering new products from agriculture. *Lowa state press, London.*

Dola, D., & Ma, M. (2023). Foliar Application Effects of Zinc Oxide Nanoparticles on Growth, Yield and Drought Tolerance of Soybean. *Bangladesh Agronomy Journal*.

Elhaj, B., Z. and Unrine, J.M. 2018. Functionalized-ZnO-nanoparticle seed treatments to enhance growth and zn content of wheat (Triticumaestivum) seedlings. *Journal of agricultural and food chemistry.* 66(46): 12166-12178.

Folch, J., Lees, M. and Sloane-Stanely, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509.

Ghasemzadeh AH, Hawa ZE, Asmah R, (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). Mol. 15: 4324-4333.

Gui, X., Dong, C., Fan, S., Jiao, C., Song, Z., Shen, J., Zhao, Y., Li, X., Zhang, F., Ma, Y., He, X., Lin, A., & Zhang, Z. (2023). Effects of CeO2 Nanoparticles on Nutritional Quality of Two Crop Plants, Corn (Zea mays L.) and Soybean (Glycine max L.). *Molecules, 28*.

Harold C. Passam, Ioannis C. Karapanos, Penelope J. Bebeli and Dimitrios Savvas.(2007). A Review of Recent Research on Tomato Nutrition, Breeding and Post-Harvest Technology with Reference to Fruit Quality, *The European Journal of Plant Science and Biotechnology* , 1(1) , 1-21.

Hedge JE, Hofreiter BT, (1962). In: Whistler RL, Be Miller JN, editors. Carbohydrate Chemistry. *New York: Academic Press.* 17: 1-19.

Horticultural Statistics at a Glance. (2018). Department of Agriculture, Cooperation & Farmers Welfare. p. 458. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm2400

Imtiaz, H., Shiraz, M.H., Rasool Mir, A., & Hayat, S. (2022). Green synthesis of nanoparticles and their effect on plant growth and development: a review. *Plant archives*.

Inmaculada yruela.(2005). Copper in plants, *Braz j plant physiology*, 17(1), , 145-156.

Itroutwar, P. D., Kasivelu, G., Raguraman, V., Malaichamy, K. & Sevathapandian, S. K. (2020). Effects of biogenic zinc oxide nanoparticles on seed germination and seedling vigor of maize (*Zea mays*). *Biocatal. Agric. Biotechnol.* 29, 101778 .

Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini, KI, (2007). *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenumgraecum*) seeds. *Food Chem.* 103: 31–37.

Khattab, S., Alkuwayti, M.A., Yap, Y., Meligy, A.M., Bani Ismail, M., & El Sherif, F. (2023). Foliar Spraying of ZnO Nanoparticals on Curcuma longa Had Increased Growth, Yield, Expression of Curcuminoid Synthesis Genes, and Curcuminoid Accumulation. *Horticulturae*.

Lowry OH, Roseobrough NJ, Farr AL, Randall RJ, (1957). Protein measurements with the Folin's phenol reagent. *J Biol Chem.* 193:265-75.

Magda Gamba, Eralda Asllanaj, Peter Francis Raguindin, Marija Glisic, Oscar H. Franco, Beatrice Minder, Weston Bussler, Brandon Metzger, Hua Kern, Taulant Muka. (2021). Nutritional and phytochemical characterization of radish (Raphanus sativus): A systematic review, *Trends in Food Science & Technology,* Volume 113,2021,Pages 205-218,ISSN 0924-2244, https://doi.org/10.1016/j.tifs.2021.04.045

melpat.com.au/documents/TechNote_CorrectingCopperDeficiencies.

Mittal ,Deepti, Kaur, Gurjeet, Singh,Parul , Yadav,Karmveer and Ali,Syed Azmal.(2022). Nanoparticle-Based Sustainable Agriculture and Food Science: Recent Advances and Future Outlook. *Frontiers in Nanotechnology* . UR - https://www.frontiersin.org/articles/10.3389/fnano.2020.579954 . VL-2(2673-3013)

Munir, T. (2018). Effect of zinc oxide nanoparticles on the growth and Zn uptake in wheat (*Triticum aestivum* L.) by seed priming method. *Digest J. Nanomater. Biostruct.* 13, 315 .

Nagar, Niharika and Devra, Vijay. (2018). Green synthesis and characterization of copper nanoparticles using Azadirachta indica leaves . *Materials Chemistry and Physics* VL - 213 SP - 44 EP - 51. DO - https://doi.org/10.1016/j.matchemphys.2018.04.007 UR - https://www.sciencedirect.com/science/article/pii/S0254058418302694

Neme K, Nafady A, Uddin S and Tola YB. (2021). Application of nanotechnology in agriculture, postharvest loss reduction and food processing: food security implication and challenges. *Heliyon. 7(12)*. https://doi.org/10.1016/j.heliyon.2021.e08539

Nene YL (1999) Utilizing traditional knowledge in agriculture. *Traditional knowledge system of India and Sri Lanka*, pp 32–38.

Nguyen, T.D., Nguyen, H.T., Nguyen, N.K., Phung, H.T., & Hoang, Y.H. (2022). Green synthesis of highly pure copper nanoparticles under microwave irradiation against pathogenic fungi on plants. *Chemical Papers, 76*, 4159 - 4167.

Ortega-Ortíz, H., Gaucin-Delgado, J.M., Preciado-Rangel, P., Fortis Hernández, M., Hernández-Montiel, L.G., Cruz-Lázaro, E.D., & Lara-Capistrán, L. (2022). Copper oxide nanoparticles biosynthetized improve germination and bioactive compounds in wheat sprouts. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*.

Patil, B.M., Patil, V.L., Ingavale, D., Bagade, C.S., Bhosale, S.R., Bhosale, R.R., P.D.Shirgave, Kamble, P.D., Patil, P.S., & Vhanalkar, S.A. (2023). Effect of ZnO nanoparticles based fertilizer on the growth parameters of maize and wheat plants.

Raghuramulu, N., Madhavan, N. K. and Kalyanasundaram, S. (2003). A Manual of Laboratory Techniques, pp. 56-58. National Institute of Nutrition. *Indian Council of Medical Research, Hyderabad, India.*

Raskar, S.V. and Laware, S.L., 2014. Effect of zinc oxide nanoparticles on cytology and seed germination in onion. *International Journal of Current Microbiology and Applied Science*.3(2): 467-473.

Rastogi Anshu, Zivcak Marek, Sytar Oksana, Kalaji Hazem M., He Xiaolan, Mbarki Sonia, Brestic Marian. (2017). Impact of Metal and Metal Oxide Nanoparticles on Plant: A Critical Review. *Frontiers in Chemistry,* VOLUME=5 URL=https://www.frontiersin.org/articles/10.3389/fchem.2017.00078 DOI=10.3389/fchem.2017.00078

Rathore, K., & Sharma, K. (2018). Biological synthesis of copper nanoparticles and their antimicrobial properties: a review.

Sandhi, R.K., Sidhu, S.K., Sharma, A., Chawla, N. and Pathak, M. (2017). Morphological and biochemical basis of resistance in okra to cotton jassid, Amrasca biguttula biguttula (Ishida). *Phytoparasitica*. 45 : 381- 394.

Sawant AA, Hegde NV, Straley BA, Donaldson SC, Love BC, Knabel SJ, Jayarao BM (2007). Antimicrobial-resistant enteric bacteria from dairy cattle. *Appl Environ Microbiol* 73:156–163.

Senthamarai, M. D. & Malaikozhundan, B. (2022). Synergistic action of zinc oxide nanoparticle using the unripe fruit extract of Aegle marmelos (L.)—Antibacterial, antibiofilm, radical scavenging and ecotoxicological effects. *Mater. Today Commun.* 30, 103228.

Singh, P., Chauhan, V., Brahmkumar, B.T., Chauhan, S.S., Simon, S., Bilal, S. and Abidi, A.B. (2014). An overview on okra [Abelmoschus esculentus (L.) Moench] and its importance as a nutritive vegetable in the world. *International Journal of Pharmacy and Biological Sciences*. 4 : 227-233.

TaheriMelika, Ataiei, Q., Ataei, Q., & YoosefiMahdieh (2015). The Effects of Zinc-Oxide Nanoparticles on Growth Parameters of Corn (SC704). *Journal of The Surface Finishing Society of Japan, 1*, 17-20.

The Hindu (2011) About 70 per cent Indians live in rural areas: Census report. http://www.thehindu.com/news/national/about70percentindiansliveinruralareascensusreport/article2230211.ece. Accessed 24 Jul 2015.

Umavathi, S., Mahboob, S., Govindarajan, M., Al-Ghanim, K.A., Ahmed, Z., Virik, P., Al-Mulhm, N., Subash, M., Gopinath, K.A., & Kavitha, C. (2020). Green synthesis of ZnO nanoparticles for antimicrobial and vegetative growth applications: A novel approach for advancing efficient high quality health care to human wellbeing. *Saudi Journal of Biological Sciences, 28*, 1808 - 1815.

Vibitha, B.V., Anitha, B., & Tharayil, N. (2020). Green synthesis of ZnO: CuO nanocomposites by Aloe Barbadansis leaf extract: Structure and photo catalytic properties.

Yruela, I. (2009). Copper in plants: acquisition, transport and interactions. *Functional plant biology : FPB, 36 5*, 409-430 .

Zafar, S., Hasnain, Z., Aslam, N., Mumtaz, S., Jaafar, H.Z., Wahab, P.E., Qayum, M., & Ormenisan, A.N. (2021). Impact of Zn Nanoparticles Synthesized via Green and Chemical Approach on Okra (Abelmoschus esculentus L.) Growth under Salt Stress. *Sustainability*.